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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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pharmaceutical combination useful for stem cell mobilization

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PHARMACEUTICAL COMBINATION USEFUL FOR STEM CELL MOBILIZATION

FIELD OF THE INVENTION

This invention regards a combination of biologically active molecules for use in the mobilization of blood stem cells in a patient or subject in need thereof. More specifically, the invention provides a combination of G-CSF and PlGF particularly effective in stimulating the mobilization of peripheral blood progenitor cells thereby increasing feasibility and efficacy of organ or cell transplantation and of chemo-radiotherapy protocols in tumor patients.

BACKGROUND OF THE INVENTION

Mobilized PBPCs represent the preferred stem cell source for HLA-matched SCT and the unique source for HLA-mismatched allografts[6,7,8,9,10,11] which is a potentially curative therapy for patients with high-risk leukemias lacking an HLA-matched related or unrelated donor, i.e., approximately 40% of the global population of patients who may benefit of allogeneic transplantation.

Protocols used to mobilize autologous PBPCs in cancer patients include the use of myeloid growth factors alone or during recovery from cytotoxic chemotherapy, with the latter approach allowing optimal PBPC mobilization[12,13,14]. Mobilization of allogeneic PBPCs from healthy donors is usually achieved by short courses of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in doses ranging from 10 to 20 μg/kg/day[15,16,17,18].

Cancer patients autografted with $\geq 5 \times 10^6$ CD34+ cells/kg experience prompt and durable hematopoietic engraftment, whereas those receiving $\leq 2 \times 10^6$ CD34+ cells/kg are at risk for delayed engraftment, engraftment failure or secondary myelodysplasia[19]. Therefore, in the setting of autologous SCT, the

availability of adequate amounts of CD34+ cells represents an essential prerequisite. Either due to prior extensive chemo-radiotherapy or disease-related factors, a substantial proportion of chemotherapy naïve (10 to 20%) or relapsed/refractory (30 to 40%) cancer patients fail to mobilize optimal amounts of CD34+ cells[20,21,22].

The collection of adequate numbers of allogeneic CD34+ cells does not represent a critical issue in recipients of HLA-identical transplants; however, 5 to 15% of normal donors experience poor stem cell mobilization and require increased doses of rhG-CSF and multiple apheretic procedures[23,24,25].Recipients of HLA-mismatched allografting require the reinfusion of "mega" doses of T-lymphocyte-depleted CD34+ cells to prevent graft failure and severe GvHD[26]. Under the standard mobilization regimen, (i.e., a 7 day course of rhG-CSF) donors for HLA-mismatched SCT undergo an average of 4 leukaphereses to collect the target cell dose of CD34+ cells (12×10^6 $CD34^+$ cells/kg body weight), with a substantial proportion of donors (20 to 25%) failing to provide the target CD34+ cell dose.

Despite age, sex, schedule of cytokine treatment as well as previous chemo-radiotherapy may affect stem cell mobilization[27,28,29], no specific characteristics have been clearly identified as predictive factors for cytokine mobilization. Therefore, any procedure applicable to cancer patients or normal donors, and capable of increasing the yield of circulating progenitors in the absence of added toxicity, is expected to have a profound impact on the feasibility, toxicity and costs both autologous and allogeneic SCT.

Increased PBPC mobilization might be achieved by using molecules capable of interfering with the mechanism(s) regulating hematopoietic stem cell trafficking, i.e., transmigration through the luminal endothelium to extravascular bone marrow spaces in homing and the reverse in mobilization[30,31,32,33]. One additional approach to enhance PBPC mobilization

relies on the use of combinations of cytokines, such as recombinant human (rh) granulocyte-macrophage colony-stimulating factor (rhGM-CSF) plus rhG-CSF[34], interleukin-3 (rhIL-3) plus rhG-CSF or rhGM-CSF[35], and PIXY-321[36]. Finally, enhancement of PBPC mobilization might be achieved by incorporating in the standard mobilization regimen early-acting cytokines, such as stem cell factor (rhSCF)[37,38] of flt-3[39] ligand, capable of expanding marrow progenitors, thus increasing the number of cells susceptible to subsequent mobilization by rhG-CSF.

So far, substitutes or adjuncts to rhG-CSF either failed to substantially improve the mobilization of blood progenitors achieved with rhG-CSF alone, or resulted in a limited improvement outweighed by a substantially increased toxicity.

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family and functions as an angiogenic amplifier by signaling through VEGF receptor-1 (VEGFR1). Recently, administration of an adenoviral vector expressing human (h) PlGF has been shown to exert complex hematopoietic effects, including enhancement of bone marrow recovery following myelosuppression, and mobilization of hematopoietic progenitors. However, the administration of growth factors following injection of recombinant adenoviral vectors presents several major differences from the direct injection of a purified factor, and might not be predictive of its effects when administered according to the modalities used in the clinical setting.

DESCRIPTION OF THE INVENTION

Due to the relevant clinical impact of any procedure capable to improve stem cell mobilization, we tested the mobilizing activity of PlGF in a mouse model allowing to simulate PBPC mobilization as occurring in a clinical situation. Normal BALB/c mice were injected intraperitoneally (IP) for 5 days

with either control vehicle (PBS/MSA), rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with either recombinant murine (rm)PlGF (2.5 – 5 μg/d) or recombinant human (rh)PlGF (5 – 10 μg/d). Blood samples were collected 2 hours after the last injection of cytokines and the following parameters were evaluated: white blood cell (WBC) counts, frequency and absolute numbers of colony-forming cells (CFC), absolute numbers of long-term culture-initiating cells (LTC-IC).

The effects of rmPlGF are illustrated in Tables 1 – 4 below. It is evident that rmPlGF injected alone has no effect on the mobilization of WBC, CFC, and LTC-IC. A 5-day injection of rmPlGF (5 μg/d) combined with rhG-CSF significantly increases mobilization of CFC and LTC-IC, as compared to rhG-CSF alone.

Tables 5 – 8 summarize the mobilizing effects of rhPlGF. Again, rhPlGF has no effects on circulating WBC or hematopoietic progenitors when injected alone. In contrast, the combined injection of rhPlGF and rhG-CSF significantly increases mobilization of CFC and LTC-IC, as compared to rhG-CSF alone.

We finally tested the mobilizing effects of a 12-day treatment with rhPlGF (10 μg/d) and rhG-CSF (10 μg/d). Mice receiving the 12-day treatment were analyzed on days 5, 8, 10, and 12 of therapy. As compared to rhG-CSF alone, the combined rhPlGF/rhG-CSF treatment significantly increased the frequency and the absolute number of blood CFC at each time-point analyzed in our study (Tables 9 – 11).

The above-indicated studies have been carried out using procedures and conditions that closely resemble the administration of hematopoietic growth factors to human patients. The results clearly demonstrate the presence of a synergistic effect by hG-CSF and hPlGF in the mobilization of peripheral blood progenitor cells.

Object of the invention is therefore a combined preparation of G-CSF and PlGF useful for stimulating blood stem cell mobilization in a patient or subject in need thereof. As used herein the terms "patient" and "subject" preferably refer to human individuals, but they may also refer to animals, especially mammals. Examples of states, conditions or diseases that may benefit from the mobilization of blood stem cells include, but are not limited to, organ or cell transplantation and tumor chemo-radiotherapy, in particular autologous[1,2] or allogeneic stem cell transplantation in patients with non-Hodgkin lymphoma (NHL), relapsed Hodgkin lymphoma (HL)[4], multiple myeloma (MM)[5], or in the recovery phase following myelosuppressive chemotherapy.

The active ingredients of the combined preparation can be simultaneously or separately administered in formulation with pharmaceutically acceptable vehicles and excipients. The parenteral route of administration is preferred. Methods for the preparation of pharmaceutical compositions suitable for parenteral administration are known in the art; details can be found in "Remington: The Science and Practice of Pharmacy", Mack Publishing Co. The amount of active ingredients in the combined preparations according to the invention can be varied depending for instance on the administration route, on the effect sought or condition to be treated, and on the response of the patient. As a general rule, an effective amount of G-CSF and PlGF is able to produce the desired response in terms of blood stem cell mobilization. The patient/subject response can be monitored during the treatment, e.g. by counting the circulating blood stem cells, and if necessary the dosages can be modified accordingly. In a preferred embodiment of the invention, recombinant hG-CSF and hPlGF are used in form of injectable solutions supplying a daily amount of the active comprised from 5 to 20 μg/kg G-CSF and from 20 to 150 μg/kg PlGF.

The following examples further illustrate the invention.

MATERIALS AND METHODS

Animals. Six- to 8-week-old female BALB/c mice, with body weight of 20 to 25 g, were purchased from Charles River (Milano, Italy, EU). Experimental procedures performed on animals were carried out in accordance with the guidelines of the United Kingdom Coordinating Committee on Cancer Research (UK Coordinating Committee on Cancer Research. UKCCCR guidelines for the welfare of animals in experimental neoplasia. Br. J. Cancer., 58:109-113, 1998.). The mice were injected daily, intraperitoneally (IP), for 5 days with either control vehicle (PBS/MSA), rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with either recombinant murine (rm)PlGF (2.5 – 5 μg/d) or recombinant human (rh)PlGF (5 – 10 μg/d). Each experiment was performed at least on three separate occasions, and three to four mice per group per time point were used.

Cytokines. Recombinant human granulocyte colony-stimulating factor (rhG-CSF, Neupogen®) was from Roche (Milan, Italy, EU); rmPlGF was purchased from R&D Systems Inc., Abingdon, United Kingdom); rhPlGF was provided from Geymonat SpA (Anagni, Italy, EU).

Mobilization protocols. The standard mobilization protocol included treatment of BALB/c with rhG-CSF (10 μg/mouse, IP) once daily for 5 days. To evaluate the mobilizing effects of PlGF, rmPlGF (2.5 – 5 μg/mouse, IP) or rhPlGF (5 – 10 μg/ mouse, IP) were administered once daily for 5 days either as a single agent or in combination with rhG-CSF. The mobilizing effects of rhPlGF were also tested by a 12-day treatment with rhPlGF (10 μg/mouse/day) and rhG-CSF (10 μg/mouse/day). Controls were injected with PBS/MSA.

Mobilization parameters. Mobilization was evaluated by white blood cell (WBC) counts, frequency and absolute numbers of colony-forming cells

(CFC), absolute numbers of long-term culture-initiating cells (LTC-IC). Unless otherwise stated, animals were sacrificed two hours after the last treatment.

Cell harvesting and separation. PB was harvested from the orbital plexus into heparin-containing tubes. After white blood cell (WBC) counting, PB was diluted (1:4, v/v) with PBS and mononuclear cells (MNCs) were separated by centrifugation (280 g, 30 min, room temperature) on a Ficoll discontinuous density gradient. Cells were then washed twice in Iscove's modified Dulbecco's medium (IMDM, Seromed, Berlin, Germany, EU) supplemented with 10% fetal bovine serum (FBS, Stem Cell Technologies, Vancouver, Canada), 2 mM L-glutamine and antibiotics.

WBC counts. WBC counts were performed using heparin-anticoagulated blood and an automated counter (ADVIA 120, Bayer, Milano, Italy, EU).

Colony-forming cell (CFC) assay. Total colony-forming cells (CFCs), i.e., granulocyte-macrophage colony-forming units (CFU-GM), erythroid burst-forming units (BFU-E), and multilineage CFU (CFU-GEMM) were assessed in standard methylcellulose cultures. Briefly, 1-ml aliquots of blood (5×10^4 to 2×10^5 MNCs) were plated in 35-mm Petri dishes in methylcellulose-based medium (HCC-3434; Stem Cell Technologies) supplemented with recombinant mouse (rm) stem cell factor (rmSCF, 50 ng/ml), mouse rm interleukin-3 (rmIL-3, 10 ng/ml), recombinant human (rh) interleukin-6 (rhIL-6, 10 ng/ml) and rh erythropoietin (rhEpo, 3 U/ml). Colonies were scored according to standard criteria after 12-14 days of incubation at 37°C in a humidified atmosphere of 5% CO_2 in air (Humphries, R.K.et al., Blood, 53:746-763, 1979.).

Long-term culture-initiating cell (LTC-IC) assay. LTC-IC were assessed in bulk cultures (Carlo-Stella C, et al. Blood. 1999;93:3973-82). Briefly, test cells ($5 - 8 \times 10^6$) were resuspended in complete medium (Myelocult™ 5100, Stem Cell Technologies) and seeded into cultures

containing a feeder layer of irradiated (2,000 cGy) murine AFT024 cells (kindly provided by Dr. K. Moore, Princeton University, Princeton, NJ, USA) (Moore KA, et al., Blood. 1997;89:4337-47).

Complete medium consisted of alpha-medium supplemented with FBS (12.5%), horse serum (12.5%), L-glutamine (2 mM), 2-mercaptoethanol (10^{-4} M), inositol (0.2 mM), folic acid (20 μM) plus freshly dissolved hydrocortisone (10^{-6} M). Cultures were fed weekly by replacement of half of the growth medium with fresh complete medium. After 4 weeks in culture, nonadherent cells and adherent cells harvested by trypsinization were pooled, washed, and assayed together for clonogenic cells in methylcellulose cultures. The total number of clonogenic cells (i.e., CFU-GEMM plus BFU-E plus CFU-GM) present in 4-week-old LTC provides a relative measure of the number of LTC-IC originally present in the test suspension. Absolute LTC-IC values were calculated by dividing the total number of clonogenic cells by 4, which is the average output of clonogenic cells per LTC-IC (Sutherland HJ, et al.,Blood. 1989;74:1563-70).

EXAMPLE 1

Table 1 – WBC counts in mice treated with rmPlGF and/or rhG-CSF

Mobilization Regimen*	WBC/μL blood	
	Median (range)	Mean ± SD
PBS/MSA	2,000 (850 – 4,000)	2,165 ± 929
rhG-CSF (10 μg/d)	6,000 (5,200 – 21,650)	9,577 ± 5,575
rmPlGF (5 μg/d)	2,450 (1,350 – 2,950)	2,450 ± 141
rhG-CSF (10 μg/d) + rmPlGF (2.5 μg/d)	5,600 (4,600 – 13,700)	7,040 ± 3,778
rhG-CSF (10 μg/d) + rmPlGF (5 μg/d)	9,500 (4,800 – 18,400)	9,980 ± 5,715

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with rmPlGF (2.5 – 5 μg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF.

EXAMPLE 2

Table 2 – Frequency of circulating CFCs in mice treated with rmPlGF and/or rhG-CSF

Mobilization Regimen*	CFCs/10^5 MNCs	
	Median (range)	Mean ± SD
PBS/MSA	7 (2 – 15)	8 ± 3
rhG-CSF (10 µg/d)	76 (51 – 148)	82 ± 29
rmPlGF (5 µg/d)	8 (7 – 9)	8 ± 1
rhG-CSF (10 µg/d) + rmPlGF (2.5 µg/d)	115 (93 – 184)	130 ± 37
rhG-CSF (10 µg/d) + rmPlGF (5 µg/d)	195 (113 – 253)	180 ± 58

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 µg/d), or a combination of rhG-CSF (10 µg/d) with rmPlGF (2.5 – 5 µg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF. CFCs include granulocyte-macrophage CFC (CFU-GM), erythroid burst-forming unit (BFU-E), and multipotent CFC (CFU-Mix). CFC data are derived from quadruplicate cultures on samples from each animal.

EXAMPLE 3

Table 3 – Absolute number of circulating CFCs in mice treated with rmPlGF and/or rhG-CSF

Mobilization Regimen*	CFCs per ml Blood	
	Median (range)	Mean ± SD
PBS/MSA	57 (9 – 288)	81 ± 75
rhG-CSF (10 µg/d)	3,129 (1,042 – 5,518)	2,977 ± 1,126
rmPlGF (5 µg/d)	96 (87 – 105)	96 ± 13
rhG-CSF (10 µg/d) + rmPlGF (2.5 µg/d)	2,568 (1,480 – 5,885)	3,198 ± 1,928
rhG-CSF (10 µg/d) + rmPlGF (5 µg/d)	6,143 (2,486 – 11,520)	6,015 ± 3,674

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 µg/d), or a combination of rhG-CSF (10 µg/d) with rmPlGF

(2.5 – 5 μg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF. CFCs include granulocyte-macrophage CFC (CFU-GM), erythroid burst-forming unit (BFU-E), and multipotent CFC (CFU-Mix). CFC data are derived from quadruplicate cultures on samples from each animal. The absolute number of circulating CFCs in blood is a function of the frequency of CFC multiplied by the total number of MNCs per ml blood.

EXAMPLE 4

Table 4 – Absolute number of circulating LTC-ICs in mice treated with rmPlGF and/or rhG-CSF

Mobilization Regimen*	LTC-ICs per ml Blood	
	Median (range)	Mean ± SD
PBS/MSA	7 (3 – 29)	9 ± 5
rhG-CSF (10 μg/d)	194 (57 – 337)	208 ± 98
rmPlGF (5 μg/d)	4 (3 – 5)	4 ± 2
rhG-CSF (10 μg/d) + rmPlGF (2.5 μg/d)	565 (279 - 852)	565 ± 405
rhG-CSF (10 μg/d) + rmPlGF (5 μg/d)	1,173 (852 – 2,070)	1,365 ± 364

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with rmPlGF (2.5 – 5 μg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF. The absolute number of circulating LTC-IC was assayed in bulk cultures. Test cells (5 - 8 x 10^6) were seeded into cultures containing a feeder layer of irradiated murine AFT024 cells. After 4 weeks in culture, nonadherent cells and adherent cells harvested by trypsinization were pooled, washed, and assayed together for clonogenic cells. The total number of clonogenic cells (i.e., CFU-Mix plus BFU-E plus CFU-GM) present in 4-week-old LTC provides a relative measure of the number of LTC-IC originally present in the test suspension. The absolute number of circulating LTC-ICs in blood is a function of the frequency of LTC-ICs multiplied by the total

number of MNCs per ml blood.

EXAMPLE 5

Table 5 – WBC counts in mice treated with rhPlGF and/or rhG-CSF

Mobilization Regimen*	WBC/μL blood	
	Median (range)	Mean ± SD
PBS/MSA	2,000 (850 – 4,000)	2,165 ± 929
rhG-CSF (10 μg/d)	6,000 (5,200 – 21,650)	9,577 ± 5,575
rhPlGF (10 μg/d)	1,900 (1,050 – 5,000)	2,296 ± 1,235
rhG-CSF (10 μg/d) + rhPlGF (5 μg/d)	14,400 (11,000 – 14,600)	13,333 ± 2,023
rhG-CSF (10 μg/d) + rhPlGF (10 μg/d)	12,800 (5,100 – 17,350)	11,728 ± 4,968

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with rhPlGF (5 – 10 μg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF.

EXAMPLE 6

Table 6 – Frequency of circulating CFCs in mice treated with rhPlGF and/or rhG-CSF

Mobilization Regimen*	CFCs/10^5 MNCs	
	Median (range)	Mean ± SD
PBS/MSA	7 (2 – 15)	8 ± 3
rhG-CSF (10 μg/d)	76 (51 – 148)	82 ± 29
rhPlGF (10 μg/d)	9 (6 – 21)	10 ± 4
rhG-CSF (10 μg/d) + rhPlGF (5 μg/d)	228 (208 – 237)	224 ± 14
rhG-CSF (10 μg/d) + rhPlGF (10 μg/d)	264 (111 – 384)	256 ± 77

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with rmPlGF (2.5

– 5 µg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF. CFCs include granulocyte-macrophage CFC (CFU-GM), erythroid burst-forming unit (BFU-E), and multipotent CFC (CFU-Mix). CFC data are derived from quadruplicate cultures on samples from each animal.

EXAMPLE 7

Table 7 – Absolute number of circulating CFCs in mice treated with rhPlGF and/or rhG-CSF

Mobilization Regimen*	CFCs per ml Blood	
	Median (range)	Mean ± SD
PBS/MSA	57 (9 – 288)	81 ± 75
rhG-CSF (10 µg/d)	3,129 (1,042 – 5,518)	2,977 ± 1,126
rhPlGF (10 µg/d)	74 (12 – 236)	82 ± 64
rhG-CSF (10 µg/d) + rhPlGF (5 µg/d)	9,467 (7,514 – 11,325)	9,435 ± 1,906
rhG-CSF (10 µg/d) + rhPlGF (10 µg/d)	11,584 (8,105 – 17,408)	12,122 ± 2,788

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 µg/d), or a combination of rhG-CSF (10 µg/d) with rmPlGF (2.5 – 5 µg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF. CFCs include granulocyte-macrophage CFC (CFU-GM), erythroid burst-forming unit (BFU-E), and multipotent CFC (CFU-Mix). CFC data are derived from quadruplicate cultures on samples from each animal. The absolute number of circulating CFCs in blood is a function of the frequency of CFC multiplied by the total number of MNCs per ml blood.

EXAMPLE 8

Table 8 – Absolute number of circulating LTC-ICs in mice treated with rhPlGF and/or rhG-CSF

Mobilization Regimen*	LTC-ICs per ml Blood	
	Median (range)	Mean ± SD
PBS/MSA	7 (3 – 29)	9 ± 5
rhG-CSF (10 µg/d)	194 (57 – 337)	208 ± 98
rhPlGF (10 µg/d)	ND	ND
rhG-CSF (10 µg/d) + rhPlGF (5 µg/d)	ND	ND
rhG-CSF (10 µg/d) + rhPlGF (10 µg/d)	1,776 (1,407 – 1,990)	1,724 ± 294

ND, not done

* BALB/c mice were injected IP for 5 days with either PBS/MSA, rhG-CSF alone (10 µg/d), or a combination of rhG-CSF (10 µg/d) with rmPlGF (2.5 – 5 µg/d). Blood samples were collected 2 hours after the last injection of rmPlGF and/or rhG-CSF. The absolute number of circulating LTC-IC was assayed in bulk cultures. Test cells (5 - 8 x 10^6) were seeded into cultures containing a feeder layer of irradiated murine AFT024 cells. After 4 weeks in culture, nonadherent cells and adherent cells harvested by trypsinization were pooled, washed, and assayed together for clonogenic cells. The total number of clonogenic cells (i.e., CFU-Mix plus BFU-E plus CFU-GM) present in 4-week-old LTC provides a relative measure of the number of LTC-IC originally present in the test suspension. The absolute number of circulating LTC-ICs in blood is a function of the frequency of LTC-ICs multiplied by the total number of MNCs per ml blood.

EXAMPLE 9

Table 9 – WBC counts in mice receiving a 12-day treatment with rhPlGF (10 μg/d) and/or rhG-CSF (10 μg/d)

Mobilization Regimen*	WBC/μL blood
	Mean ± SD
PBS/MSA	2,165 ± 929
5-day rhG-CSF	18,683 ± 3,001
5-day rhG-CSF + rhPlGF	16,083 ± 1,227
8-day rhG-CSF	22,017 ± 5,778
8-day rhG-CSF + rhPlGF	16,000 ± 6,354
10-day rhG-CSF	21,500 ± 3,317
10-day rhG-CSF + rhPlGF	24,800 ± 6,699
12-day rhG-CSF	43,100 ± 8,598
12-day rhG-CSF + rhPlGF	46,167 ± 5,678

* BALB/c mice were injected IP for 12 days with either PBS/MSA, rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with rhPlGF (10 μg/d). Blood samples were collected after 5, 8, 10, and 12 days of treatment.

EXAMPLE 10

Table 10 – Frequency of circulating CFCs in mice receiving a 12-day treatment with rhPlGF (10 μg/d) and/or rhG-CSF (10 μg/d)

Mobilization Regimen*	CFCs/10^5 MNCs
	Mean ± SD
PBS/MSA	8 ± 3
5-day rhG-CSF	63 ± 12
5-day rhG-CSF + rhPlGF	297 ± 80
8-day rhG-CSF	70 ± 5
8-day rhG-CSF + rhPlGF	180 ± 20
10-day rhG-CSF	102 ± 8
10-day rhG-CSF + rhPlGF	274 ± 34
12-day rhG-CSF	106 ± 19
12-day rhG-CSF + rhPlGF	299 ± 49

* BALB/c mice were injected IP for 12 days with either PBS/MSA, rhG-CSF alone (10 μg/d), or a combination of rhG-CSF (10 μg/d) with rhPlGF (10 μg/d). Blood samples were collected after 5, 8, 10, and 12 days of treatment. CFCs include granulocyte-macrophage CFC (CFU-GM), erythroid burst-forming unit (BFU-E), and multipotent CFC (CFU-Mix). CFC data are derived from quadruplicate cultures on samples from each animal.

EXAMPLE 11

Table 1i – Absolute number of circulating CFCs in mice receiving a 12-day treatment with rhPlGF (10 µg/d) and/or rhG-CSF (10 µg/d)

Mobilization Regimen*	CFCs per ml Blood
	Mean ± SD
PBS/MSA	81 ± 75
5-day rhG-CSF	3,427 ± 232
5-day rhG-CSF + rhPlGF	11,649 ± 1,827
8-day rhG-CSF	6,361 ± 1,931
8-day rhG-CSF + rhPlGF	10,341 ± 799
10-day rhG-CSF	4,335 ± 923
10-day rhG-CSF + rhPlGF	14,104 ± 2,687
12-day rhG-CSF	10,968 ± 2,183
12-day rhG-CSF + rhPlGF	32,024 ± 4,915

* BALB/c mice were injected IP for 12 days with either PBS/MSA, rhG-CSF alone (10 µg/d), or a combination of rhG-CSF (10 µg/d) with rhPlGF (10 µg/d). Blood samples were collected after 5, 8, 10, and 12 days of treatment. CFCs include granulocyte-macrophage CFC (CFU-GM), erythroid burst-forming unit (BFU-E), and multipotent CFC (CFU-Mix). CFC data are derived from quadruplicate cultures on samples from each animal. The absolute number of circulating CFCs in blood is a function of the frequency of CFC multiplied by the total number of MNCs per ml blood.

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CLAIMS

1. Combined pharmaceutical preparation containing G-CSF and PlGF as the active substances, for use in the mobilization of blood stem cells in a patient or subject in need thereof.

2. Combined preparation according to claim 1, wherein G-CSF and PlGF are simultaneously or separately administered to said patient or subject.

3. Combined preparation according to claims 1-2, for parenteral administration.

4. Combined preparation according to claims 1-3, containing recombinant hG-CSF and hPlGF.

5. Combined preparation according to claims 1-4, containing from 5 to 20 μg/kg G-CSF and from 20 to 150 μg/kg PlGF.

6. Use of a combination of G-CSF and PlGF in the manufacture of a pharmaceutical composition for the treatment of states, conditions or diseases that require the mobilization of blood stem cells.

7. Use according to claim 6, in the manufacture of a parenteral composition.

8. Use according to claim 6 of a combination of recombinant hG-CSF and hPlGF.

9. Use according to claim 6, wherein said states, conditions or diseases include organ or cell transplantation and tumor chemo-radiotherapy, in particular autologous or allogeneic stem cell transplantation in patients with non-Hodgkin lymphoma (NHL), relapsed Hodgkin lymphoma (HL), multiple myeloma, or the recovery phase following myelosuppressive chemotherapy.

10. Use according to claim 6, wherein the pharmaceutical composition provides a daily amount of 10 μg/kg G-CSF and of 130 μg/kg PlGF.

ABSTRACT

PHARMACEUTICAL COMBINATION USEFUL FOR STEM CELL MOBILIZATION

Combined pharmaceutical preparation containing G-CSF and PlGF as the active substances, are useful in the mobilization of blood stem cells in a patient or subject in need thereof.

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